Fish oil supplementation improves docosahexaenoic acid status of malnourished infants

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Abstract

Aim—To investigate whether the low docosahexaenoic acid (DHA) status of malnourished, mostly breast fed, Pakistani children can be improved by fish oil (FO) supplementation.

Methods—Ten malnourished children (aged 8–30 months) received 500 mg FO daily for nine weeks. The supplement contained 62.8 mol% (314 mg) long chain polyunsaturated fatty acids of the ω 3 series (LCPUFA ω 3) and 22.5 mol% (112 mg) DHA. Seven FO unsupplemented children served as controls. Red blood cell (RBC) fatty acids were analysed at baseline and at the study end.

Results—FO supplementation augmented mean (SD) RBC DHA from 2.27 (0.81) to 3.35 (0.76) mol%, without significantly affecting the concentrations of LCPUFA ω 6. Unsupplemented children showed no RBC fatty acid changes. One FO supplemented child with very low initial RBC arachidonic acid showed a remarkable increase from 4.04 to 13.84 mol%, whereas another with high RBC arachidonic acid showed a decrease from 15.64 to 10.46 mol%.

Conclusion—FO supplementation improves the DHA status of malnourished children. The supplement is apparently well absorbed and not exclusively used as a source of energy.

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Keywords: erythrocyte; fish oil; long chain polyunsaturated fatty acids; malnutrition

Essential fatty acids (EFAs) constitute a group of fatty acids that cannot be synthesised de novo. Long chain polyunsaturated fatty acids (LCPUFAs; containing at least 20 carbon atoms and at least three double bonds in the methylene interrupted configuration) derive either from the diet or from desaturation–chain elongation of the two parent EFAs linoleic (18:2 ω 6; LA) and α -linolenic (18:3 ω 3; ALA) acids. LCPUFAs are structural components of cell membrane phospholipids and precursors of eicosanoids. They play important roles in the development of the central nervous system, including the retina.^{1–2} Postnatal docosahexaenoic acid (22:6 ω 3; DHA) status is, for example, related to visual acuity^{3–6} and neurodevelopment,^{7–8} whereas arachidonic acid (20:4 ω 6; AA) has been associated with pre- and postnatal growth.^{9–10}

In a previous study we found very low erythrocyte (RBC) DHA concentrations in malnourished, mostly breast fed 4–56 month old Pakistani children.¹¹ A low breast milk LCPUFA ω 3 content was identified as the major cause of their poor DHA status.^{11a} Apart from low intake, poor PUFA status of malnourished children may also derive from malabsorption,^{12–13} impaired desaturation and elongation,^{11–14–17} peroxidation of PUFA,¹⁶ and the use of PUFA as an energy source via β oxidation.^{18–19} Many studies have shown that fish oil (FO) supplementation increases DHA content of many blood compartments, including RBC and plasma lipid fractions.^{5–20–25} It is, however, unknown whether this also applies to malnourished children, as they may have poor fat absorption, or use the supplement as an energy source. In the present study we supplemented 10 malnourished Pakistani children with 500 mg FO daily for nine weeks and investigated whether it improved their DHA status, as derived from their RBC fatty acid composition. Seven unsupplemented counterparts served as controls.

Subjects and methods

SUBJECTS, SUPPLEMENT, AND STUDY DESIGN

Seventeen infants were recruited from the Nutrition Rehabilitation Center of the Pediatric Department, Federal Government Services Hospital, Islamabad (Pakistan). Anthropometric, demographic, socioeconomic, and clinical data were documented. They were classified according to local growth charts, provided by the WHO. Grades 2 and 3 malnutrition were defined as weight for age below the mean minus two standard deviations (SDs) and three SDs, respectively, using the data from the United States National Center for Health Statistics (NCHS) as a reference. The study conformed to local ethical standards and the Helsinki declaration of 1975 as revised in 1989.

The children were randomly assigned to receive one 500 mg capsule of FO (Pikazol, Hadsund, Norway) daily for approximately nine weeks ($n = 10$), or no oil (controls; $n = 7$). As the capsules proved too big to swallow, they were pierced and the oil was given by spoon. As stated by the manufacturer, each capsule contained 1.5 IU vitamin E. Table 1 provides details of the fatty acid composition, as established by us. The daily intakes corresponded with 190 mg eicosapentaenoic acid (EPA), 112 mg DHA, and 10 mg AA. EDTA anticoagulated blood (2.5 ml at most) was taken in an undefined metabolic state at base-

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Table 1 Fatty acid composition of the fish oil capsules

$\omega 3$ series	
18:3 $\omega 3$	1.0
18:4 $\omega 3$	6.1
20:4 $\omega 3$	2.0
20:5 $\omega 3$	37.9
22:5 $\omega 3$	3.8
22:6 $\omega 3$	22.5
$\omega 6$ series	
18:2 $\omega 6$	1.4
18:3 $\omega 6$	0.4
20:2 $\omega 6$	0.3
20:3 $\omega 6$	0.3
20:4 $\omega 6$	1.9
22:4 $\omega 6$	0.2
22:5 $\omega 6$	0.5
$\omega 9$ series	
18:1 $\omega 9$	6.7
20:1 $\omega 9$	1.6
22:1 $\omega 9$	1.0
24:1 $\omega 9$	0.1
SAFA	5.9
MCSAFA	3.2
MUFA	15.8
PUFA	78.3
Sum $\omega 3$ ss	73.1
Sum $\omega 6$	5.1
Sum $\omega 7$	6.5
Sum $\omega 9$	9.3
LCPUFA $\omega 3$	66.1
LCPUFA $\omega 6$	3.2

Data represent a selection of fatty acids, expressed in mol% (mol/100 mol).

SAFA, saturated fatty acids; MCSAFA, medium chain SAFA (C6–C14); MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; LCPUFA, long chain PUFA (C $\geq$ 20, double bonds $\geq$ 3).

Table 2 Characteristics of the malnourished children

	Controls (n = 7)	Fish oil (n = 10)
Age (mth)	24 (4–56)	17 (8–30)
Weight (kg)	8.0 (4.0–10.3)	5.9 (4.3–8.3)
Grade 2/grade 3	4/3	3/7
Hb (g/l)	104 (73–122)	83 (60–109)
RBC (10 ¹² /l)	4.37 (3.91–5.05)	4.20 (3.02–5.01)
Infections*	5/7	8/10
Breast fed	4/7	4/9

Data expressed as median (range), or fraction.

*Upper respiratory and/or gastrointestinal infections.

line and at the study end. All infants received a multivitamin syrup and occasionally medical treatment, including antibiotics (mostly erythromycin). The multivitamin syrup contained vitamin B complex, vitamin C, and lysine (Actoplex-C, Atco Lab Private Ltd, Karachi, Pakistan). All mothers received nutritional education at the Nutrition Rehabilitation Center. The education included cooking lessons, information about what to feed the child, and how to prepare it. Informed consent was obtained from all mothers.

SAMPLE PROCESSING AND ANALYSIS

The blood samples were immediately cooled in melting ice. Haematological indices were measured within 24 hours by means of a Sysmex counter. The remaining volume was centrifuged at 800 *g* for 10 minutes in a cooled centrifuge. The plasma and buffy coat were removed and the RBCs were washed three times with isotonic saline. The RBCs were finally resuspended to a haematocrit of about 50% and counted again. For the analysis of RBC fatty acids, 200 μ l of this suspension was

transferred to a 15 ml Teflon stoppered tube, containing 1 mg butylated hydroxytoluene (antioxidant) and 50 μ g margaric acid (17:0; internal quantification standard). The preserved RBC samples were stored at -20°C and transported to the Netherlands in dry ice. Fatty acid measurements were performed using our previously reported methods—capillary gas chromatography with flame ionisation detection.^{26 27}

STATISTICS

Differences between RBC fatty acid content of FO supplemented infants and controls were analysed by the Mann–Whitney U test. Differences in RBC fatty acid content at baseline and at study end were analysed by the Wilcoxon signed rank test. A probability value of $p < 0.05$ was considered statistically significant. All statistics were evaluated with SPSS (SPSS 6.0 for Windows, SPSS Inc., Chicago, Illinois, USA).

Results

Table 2 presents the characteristics of the study groups. The mean FO supplementation period was 9.1 weeks (range 7–12). For the control group the mean time interval between enrolment and collection of the second blood sample was 9.7 weeks (range 8–16). Eight infants in the FO supplementation group and all controls had gained weight at the study end. When expressed as percentage of the reference average weight for age (NCHS) their median weights improved from 53.5% (range 47–68%) to 60% (46–69%) in the FO group and from 63% (57–68%) to 68% (60–70%) in the control group.

Table 3 shows selected RBC fatty acids of the children. FO supplemented children and controls did not have significantly different RBC fatty acid compositions at enrolment. The controls did not exhibit RBC fatty acid differences between enrolment and study end. Compared with controls, FO supplemented children had at study end higher RBC 20:5 $\omega 3$ ($p = 0.010$), 22:6 $\omega 3$ ($p = 0.007$), sum of $\omega 3$ fatty acids ($p = 0.007$), $\omega 3/\omega 6$ ($p = 0.010$), LCPUFA $\omega 3/\text{LCPUFA}\omega 6$ ($p = 0.002$), and 24:1 $\omega 9$ ($p = 0.043$). FO supplemented children showed increases of RBC 20:5 $\omega 3$ ($p = 0.005$), 22:5 $\omega 3$ ($p = 0.007$), 22:6 $\omega 3$ ($p = 0.009$), sum of $\omega 3$ fatty acids ($p = 0.005$), LCPUFA $\omega 3$ ($p = 0.005$), $\omega 3/\omega 6$ ($p = 0.005$), LCPUFA $\omega 3/\text{LCPUFA}\omega 6$ ($p = 0.005$), and 26:0 ($p = 0.022$, not shown) from baseline to study end. There were no changes in RBC $\omega 6$ fatty acids.

Discussion

We investigated whether the DHA status of malnourished Pakistani infants can be improved by FO supplementation. It was found that daily administration of 500 mg FO, containing 62.8 mol% LCPUFA $\omega 3$, for nine weeks resulted in about 50% increases of RBC DHA and LCPUFA $\omega 3$, without affecting the RBC LCPUFA $\omega 6$ content. The unsupplemented control group did not exhibit RBC fatty acid changes. The results of this study

Table 3 Erythrocyte fatty acids of fish oil supplemented children and controls

Fatty acids	Controls (n = 7)		Fish oil (n = 10)	
	Baseline	9 weeks	Baseline	9 weeks
$\omega 3$ series				
18:3 $\omega 3$	0.16 (0.05)	0.15 (0.05)	0.18 (0.12)	0.22 (0.14)
20:5 $\omega 3$	0.26 (0.08)	0.29 (0.17)	0.31 (0.33)	0.72 (0.42)*§
22:5 $\omega 3$	1.62 (0.35)	1.52 (0.47)	1.51 (0.75)	2.08 (0.92)†
22:6 $\omega 3$	2.11 (0.49)	2.07 (0.71)	2.27 (0.81)	3.35 (0.76)†§
$\omega 6$ series				
18:2 $\omega 6$	9.94 (1.99)	9.80 (2.24)	7.43 (1.52)	7.83 (1.99)
20:2 $\omega 6$	0.23 (0.07)	0.21 (0.10)	0.18 (0.06)	0.21 (0.07)
20:3 $\omega 6$	1.53 (0.17)	1.48 (0.17)	1.32 (0.39)	1.39 (0.29)
20:4 $\omega 6$	14.03 (1.11)	13.62 (1.73)	13.01 (3.40)	13.04 (1.49)
22:4 $\omega 6$	3.27 (0.36)	2.91 (0.46)	2.64 (0.71)	2.43 (0.48)
22:5 $\omega 6$	0.95 (0.17)	0.89 (0.18)	0.92 (0.27)	0.82 (0.17)
$\omega 9$ series				
18:1 $\omega 9$	10.95 (1.07)	11.10 (1.39)	12.90 (1.44)	12.33 (1.62)
20:1 $\omega 9$	0.22 (0.04)	0.23 (0.04)	0.25 (0.05)	0.22 (0.06)
20:3 $\omega 9$	0.35 (0.09)	0.43 (0.28)	0.60 (0.44)	0.60 (0.32)
22:3 $\omega 9$	0.18 (0.12)	0.16 (0.13)	0.29 (0.22)	0.23 (0.14)
24:1 $\omega 9$	3.66 (0.37)	3.38 (0.42)	4.08 (0.49)	3.94 (0.49)¶
SAFA	48.59 (0.66)	50.04 (3.93)	50.24 (5.13)	48.88 (1.38)
MUFA	16.78 (1.44)	16.44 (1.57)	19.14 (1.53)	18.20 (1.53)
PUFA	34.64 (1.95)	33.53 (3.75)	30.64 (5.93)	32.91 (2.17)
Sum $\omega 3$	4.16 (0.79)	4.03 (1.24)	4.26 (1.71)	6.37 (1.66)*§
Sum $\omega 6$	29.95 (1.73)	28.91 (3.34)	25.51 (5.00)	25.70 (3.13)
Sum $\omega 7$	1.91 (0.43)	1.70 (0.51)	1.83 (0.50)	1.67 (0.32)
Sum $\omega 9$	15.40 (1.41)	15.33 (1.76)	18.15 (1.96)	17.37 (2.11)
LCPUFA $\omega 3$	3.99 (0.79)	3.88 (1.22)	4.08 (1.60)	6.15 (1.54)*‡
LCPUFA $\omega 6$	19.78 (1.43)	18.90 (2.06)	17.90 (4.53)	17.68 (2.01)
$\omega 3/\omega 6$	0.14 (0.03)	0.14 (0.05)	0.17 (0.08)	0.26 (0.10)*§
L $\omega 3/\text{L}\omega 6$	0.20 (0.03)	0.20 (0.06)	0.23 (0.09)	0.35 (0.11)*‡

Data represent mean (SD) and are expressed as mol% (mol/100 mol). For abbreviations see table 1. Controls received nutritional rehabilitation for 9.7 weeks (range 8–16); the fish oil group was additionally supplemented with 500 mg fish oil (see table 1) daily for 9.1 weeks (range 7–12).

*Significant difference between baseline and 9 weeks at $p \leq 0.005$.

†Significant difference between baseline and 9 weeks at $p \leq 0.01$.

‡Significant difference between fish oil and control group at $p \leq 0.005$.

§Significant difference between fish oil and control group at $p \leq 0.01$.

¶Significant difference between fish oil and control group at $p \leq 0.05$.

show that the FO supplement is apparently well absorbed in malnourished children and that the LCPUFA ω 3 are not exclusively used as a source of energy under these conditions.

This is to our knowledge the first report to show the effects of LCPUFA supplementation on the RBC fatty acid composition of malnourished children. Koletzko *et al*¹⁷ monitored the plasma fatty acid composition of eight recovering malnourished children during treatment with a high energy and high protein diet (including maize porridge, milk, eggs, beans, fish, meat, and vegetable oils). They found a slight improvement of the essential fatty acid status after 14 days treatment without additional LCPUFA supplements.

The most remarkable changes in the RBC fatty acid composition were recorded in a severely malnourished 21 month old, almost exclusively breast fed marasmic girl. Her weight was only 5.5 kg (47% of the reference average) at enrolment and she had a "dry" skin. After nine weeks FO supplementation she had gained 1.8 kg (to reach 60% of the reference average). There was no clinical evidence that this weight gain was caused by water retention. Concomitantly she displayed an increase of both RBC ω 3 and ω 6 fatty acids, mainly at the expense of the sum of the saturated fatty acids (from 65.39 to 48.24 mol%). RBC DHA increased from 0.41 to 2.50 mol%. Surprisingly, a large increase was on account of RBC AA (from 4.04 to 13.84 mol%). It seems unlikely that this increase can be explained by the low AA intake from the supplement (10 mg per day). The RBC 18:2 ω 6/20:4 ω 6 ratio (a parameter of combined activities of Δ 6-desaturase, chain elongation, and Δ 5-desaturase) decreased from 1.60 to 0.71 mol/mol, while the RBC 20:3 ω 6/20:4 ω 6 ratio (a parameter of Δ 5-desaturase activity) did not change. The data of this child suggest low activity of Δ 6-desaturase at enrolment, which subsequently improved during the intervention period. A positive effect of ω 3 fatty acids on LCPUFA ω 6 fatty acids has previously been reported by Bjerve *et al*.²⁵ They observed an increase of plasma and RBC LCPUFA ω 6 in patients with ω 3 fatty acid deficiency following FO supplementation. It was suggested that ω 6 fatty acids cannot accumulate normally in cell membranes at the condition of low ω 3 fatty acid supply. In contrast to the above case, a 30% decrease of RBC AA was observed in a 16 month old girl with a relatively high RBC AA level at baseline (from 15.64 to 10.46 mol%). Reduction of plasma and RBC AA concentrations after FO supplementation has previously been noticed in preterm^{21,22} and term⁵ infants and adults.²⁰ The contradictory findings in the two cases may indicate that the effect of FO supplementation on RBC AA concentrations is dependent on baseline RBC AA contents, causing increases at low initial RBC AA status, and decreases at high initial RBC AA status.

We conclude that FO supplementation of malnourished Pakistani children improves their DHA status. Replacement of the presently employed purified FO by the much cheaper cod liver oil (approximately 10%

DHA) may give similar results. Another factor in favour of cod liver oil as a supplement would be its high vitamin A and D contents. Malnourished children often have low vitamin A and D status.^{12,28} Further investigations are needed to clarify whether AA supplementation should be recommended in addition to DHA supplementation to prevent any adverse effects on the LCPUFA ω 6 status that are caused by augmentation of the LCPUFA ω 3 status.

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